#30/4 DmT 2-28-03

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

Elmore et al

Atty. Ref.: 1498-133

Serial No. 08/981,087

Group: 1647

Filed: May 27, 1998

Examiner: Turner

For: TYPE F BOTULINUM TOXIN AND USE THEREOF

* * * * * * * * * * *

January 28, 2003

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

AMENDMENT

Responsive to the Official Action dated October 28, 2002, and the Notice to Comply received with the same (copy attached), entry and consideration of the following amendments and remarks are requested.

IN THE SPECIFICATION

Amend the specification as follows.

Page 10, delete the paragraph spanning lines 16-18 and insert the following therefor:

--**Figure 3:** shows an example of a recombinant plasmid (pFHC206) made in which the synthetic DNA fragment of Figure 5 is inserted into the expression plasmid pMal-C2; (SEQ ID NOs:7 (DNA) and 8 (amino acid) and--

#1

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Box Seq.

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C# M#

Elmore et al

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Title: TYPE F BOTULINUM TOXIN AND USE THEREOF

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

RESPONSE/AMENDMENT/LETTER

This is a response/amendment/letter in the above-identified application and includes an attachment which is hereby incorporated by reference and the signature below serves as the signature to the attachment in the absence of any other signature thereon.

☒ **Correspondence Address Indication Form Attached.**

Fees are attached as calculated below:

Total effective claims after amendment **28** minus highest number previously paid for **24** (at least 20) = 4 x $ 18.00	$	72.00
Independent claims after amendment **6** minus highest number previously paid for **4** (at least 3) = 2 x $ 84.00	$	168.00
If proper multiple dependent claims now added for first time, add $280.00 (ignore improper)	$	0.00
Petition is hereby made to extend the current due date so as to cover the filing date of this paper and attachment(s) ($110.00/1 month; $410.00/2 months; $930.00/3 months)	$	0.00
Terminal disclaimer enclosed, add $ 110.00	$	0.00
☐ First/second submission after Final Rejection pursuant to 37 CFR 1.129(a) ($750.00) ☐ Please enter the previously unentered , filed ☐ Submission attached	$	0.00
Subtotal	$	**240.00**
If "small entity," then enter half (1/2) of subtotal and subtract ☐ Applicant claims "small entity" status. ☐ Statement filed herewith	-$	0.00
Rule 56 Information Disclosure Statement Filing Fee ($180.00)	$	0.00
Assignment Recording Fee ($40.00)	$	0.00
Other: Copy of Notice to Comply; Statement; Paper and Computer Readable Copies of Sequence Listing		**0.00**
TOTAL FEE ENCLOSED	$	**240.00**

The Commissioner is hereby authorized to charge any deficiency, or credit any overpayment, in the fee(s) filed, or asserted to be filed, or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Account No. 14-1140. A duplicate copy of this sheet is attached.

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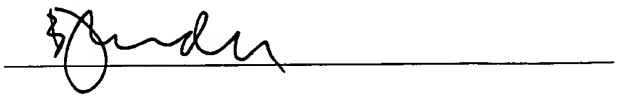
NIXON & VANDERHYE P.C.
By Atty: B. J. Sadoff, Reg. No. 36,663

Signature: 

Immunol Invest 1997 Jun;26(4):491-504 Related Articles, Links

Localizati n of the regi ns n the C-terminal d main of the heavy chain of botulinum A recognized by T lymphocytes and by antibodies after immunization of mice with pentavalent toxoid.

Rosenberg JS, Middlebrook JL, Atassi MZ.

Department of Biochemistry, Baylor College of Medicine, Houston, TX 77030, USA.

We have mapped the regions recognized by T and/or B cells (Abs) on the C-terminal domain (Hc) of the heavy chain of botulinum neurotoxin serotype A (BoNT/A) after immunization of two inbred mouse strains with pentavalent toxoid (BoNTs A, B, C, D and E). Using a set of synthetic overlapping peptides, encompassing the entire Hc domain (residues 855-1296), we demonstrated that T cells of Balb/c (H-2d) mice, primed with one injection of toxoid, recognized two major regions within residues 897-915 and 939-957. After multiple inoculations with toxoid, T cells of Balb/c expanded their recognition ability and responded very well to challenge with peptide 1261-1279 and moderately to stimulation with peptide 1149-1167. Unlike Balb/c T cells, those of toxoid-primed SJL (H-2s) mice exhibited a more complex profile and responded to challenge with a large number of overlapping peptides. After one toxoid injection, however, three peptides, 897-915, 939-957/953-971 overlap and 1051-1069, were the most potent T cells stimulators. After three toxoid injections, peptides 897-915 and 1051-1069 remained immunodominant while the third region was shifted upstream to 925-943/939-957 overlap. The immunodominant epitope within peptide 897-915 was recognized exclusively by T cells, since no Abs were detected against this region. The Ab binding profiles of the two mouse strains were quite similar, showing only small quantitative differences. Both, Balb/c and SJL anti-toxoid Abs displayed strong binding mainly to peptide 1177-1195, followed by peptides 869-887/883-901 overlap and 1275-1296. In addition, a significant amount of Balb/c anti-toxoid Abs was bound to peptide 1135-1153. Unlike Balb/c Abs, that interacted weakly with peptides 995-1013 and 1051-1069, the anti-toxoid Abs of SJL mice exhibited strong binding toward both peptides. The results showed that, in a given strain, the regions recognized by anti-toxoid Abs and T cells may coincide or may be uniquely B or T cell determinants.

Vaccine 1998 Nov;16(19):1850-6 Related Articles, Links

Identifying the principal protective antigenic determinants of type A botulinum neurotoxin.

Bavari S, Pless DD, Torres ER, Lebeda FJ, Olson MA.

Department of Cell Biology and Biochemistry, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD 21702-5011, USA.

The neurotoxins from Clostridium botulinum (BoNT serotypes A-G) exert their lethal effect by preventing the release of acetylcholine at the neuromuscular junction. As with tetanus toxin, immunization with a non-toxic fragment, the 50 kDa C-terminal portion of BoNT/A (Hc; residues 861-1296), protects mice against lethal challenges with the intact toxin. To locate the neutralizing epitopes, several protective monoclonal antibodies (mAbs) against BoNT/A-Hc were isolated and cloned. Specific binding of the mAbs to BoNT/A-Hc was demonstrated by surface plasmon resonance, with Kas in the range of 10(-10) to 10(-11) M. These antibodies recognized a genetically engineered polypeptide (1150-1289) that was previously shown to induce protective immunity. Pri r t the determinati n of the X-ray crystal structure of the tetanus neurotoxin Hc fragment, molecular modelling studies indicated that it contained two highly solvent-exposed loops. Based on these predictions, two 25-mer